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Supplemental Information

Salicylic Acid Targets Protein Phosphatase 2A to Attenuate Growth in Plants

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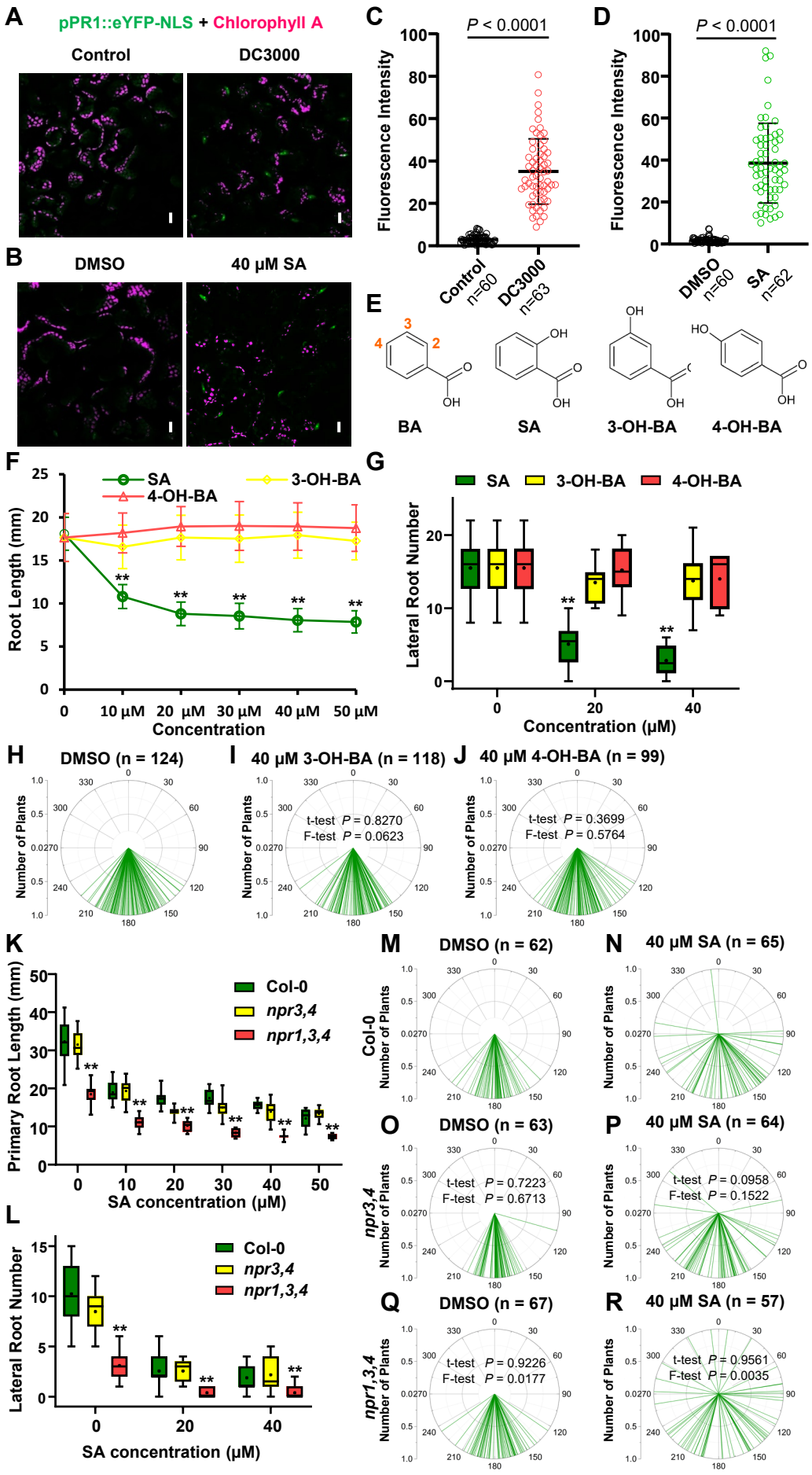

A
pPR1::eYFP-NLS + Chlorophyll A
Control
DC3000
B
DMSO
40 μM SA
C
P < 0.0001
Fluorescence Intensity
Control n=60
DC3000 n=63
D
P < 0.0001
Fluorescence Intensity
DMSO n=60
SA n=62
E
BA
SA
3-OH-BA
4-OH-BA
F
SA
4-OH-BA
3-OH-BA
Root Length (mm)
0 10 μM 20 μM 30 μM 40 μM 50 μM
Concentration
G
SA
3-OH-BA
4-OH-BA
Lateral Root Number
Concentration (μM)
H
DMSO (n = 124)
I
40 μM 3-OH-BA (n = 118)
t-test P = 0.8270
F-test P = 0.0623
J
40 μM 4-OH-BA (n = 99)
t-test P = 0.3699
F-test P = 0.5764
Number of Plants
K
Col-0
npr3,4
npr1,3,4
Primary Root Length (mm)
SA concentration (μM)
L
Col-0
npr3,4
npr1,3,4
Lateral Root Number
SA concentration (μM)
M
DMSO (n = 62)
N
40 μM SA (n = 65)
O
DMSO (n = 63)
t-test P = 0.7223
F-test P = 0.6713
P
40 μM SA (n = 64)
t-test P = 0.0958
F-test P = 0.1522
Q
DMSO (n = 67)
t-test P = 0.9226
F-test P = 0.0177
R
40 μM SA (n = 57)
t-test P = 0.9561
F-test P = 0.0035
Col-0
npr3,4
npr1,3,4
Number of Plants

Figure S1. Structural isomers and the specific role of SA in the root growth regulation. Related to Figures 1 & 2.

(A-D) Induced *pPR1::eYFP-NLS* expression by *P. syringae* DC3000 (A, C) or SA (B, D) in cotyledons. (A, C) 5-d-old *pPR1::eYFP-NLS* seedlings were treated with *P. syringae* DC3000 (OD_{600} = 0.01, ~5×10^6 CFU/mL) or with resuspension buffer (control) for 48 h, and were then imaged by CLSM. Scale bars, 10 μm. (B, D) Induced *pPR1::eYFP-NLS* expression by SA in cotyledons. 5-d-old *pPR1::eYFP-NLS* seedlings were transferred to plates with DMSO or 40 μM SA for 24h, and were then imaged by CLSM. Scale bars, 10 μm. For quantification, the average GFP fluorescence of 5-10 representative cells from 10 seedlings for each treatment was measured by Fiji. The data points were shown as dot plots. Dots represent individual values, and lines indicate mean ± SD. *P* values were calculated by a two-tailed t-test.

(E) Structures of benzoic acid analogues, including benzoic acid (BA), 2-hydroxybenzoic acid (also known as ortho-salicylic acid, SA), 3-hydroxybenzoic acid (3-OH-BA, also known as meta-salicylic acid), and 4-hydroxybenzoic acid (4-OH-BA, also known as para-salicylic acid). Chemical structures were illustrated with the ChemSketch program.

(F) 3-OH-BA and 4-OH-BA do not inhibit primary root elongation. Root length of 7-d-old Col-0 seedlings grown on MS plates containing different concentrations of SA, 3-OH-BA or 4-OH-BA was measured. Note that the same DMSO control was used for all the indicated chemicals. n = 100-129. **, $P < 0.01$, by a two-tailed t-test.

(G) 3-OH-BA and 4-OH-BA do not repress lateral root formation. The lateral root number of 10-d-old plants was counted. The same DMSO control was used for all the indicated chemicals. n = 10-22. **, $P < 0.01$, by a two-tailed t-test.

(H-J) 3-OH-BA and 4-OH-BA do not have a significant effect on root gravitropism. The root tip angles of 7-d-old Col-0 seedlings on different plates were measured. *P* values were calculated by a two-tailed t-test to evaluate the mean value and by a further F-test to indicate differences of variances.

(K) SA inhibited primary root elongation, which was not suppressed by NPR deficiency. Root length of 7-d-old Col-0, *npr3,4* and *npr1,3,4* seedlings grown on MS plates containing different concentrations of SA was measured. n = 10-26. **, $P < 0.01$. *P* values were calculated by one-way ANOVA with a Tukey multiple comparison test, compared to Col-0 in each treatment.

(L) Inhibition of lateral root formation by SA does not depend on *NPRs*. The lateral root number of 10-d-old Col-0, *npr3,4* and *npr1,3,4* plants was counted. n = 9-24. **, $P < 0.01$. *P* values were calculated by one-way ANOVA with a Tukey multiple comparison test, compared to Col-0.

(M-R) SA repressed root gravitropism independently of *NPRs*. The angles of root tips of 7-d-old Col-0 (M, N), *npr3,4* (O, P) and *npr1,3,4* (Q, R) seedlings were measured. *P* values were calculated by a two-tailed t-test to evaluate the mean value and by a further F-test to indicate differences of variances.

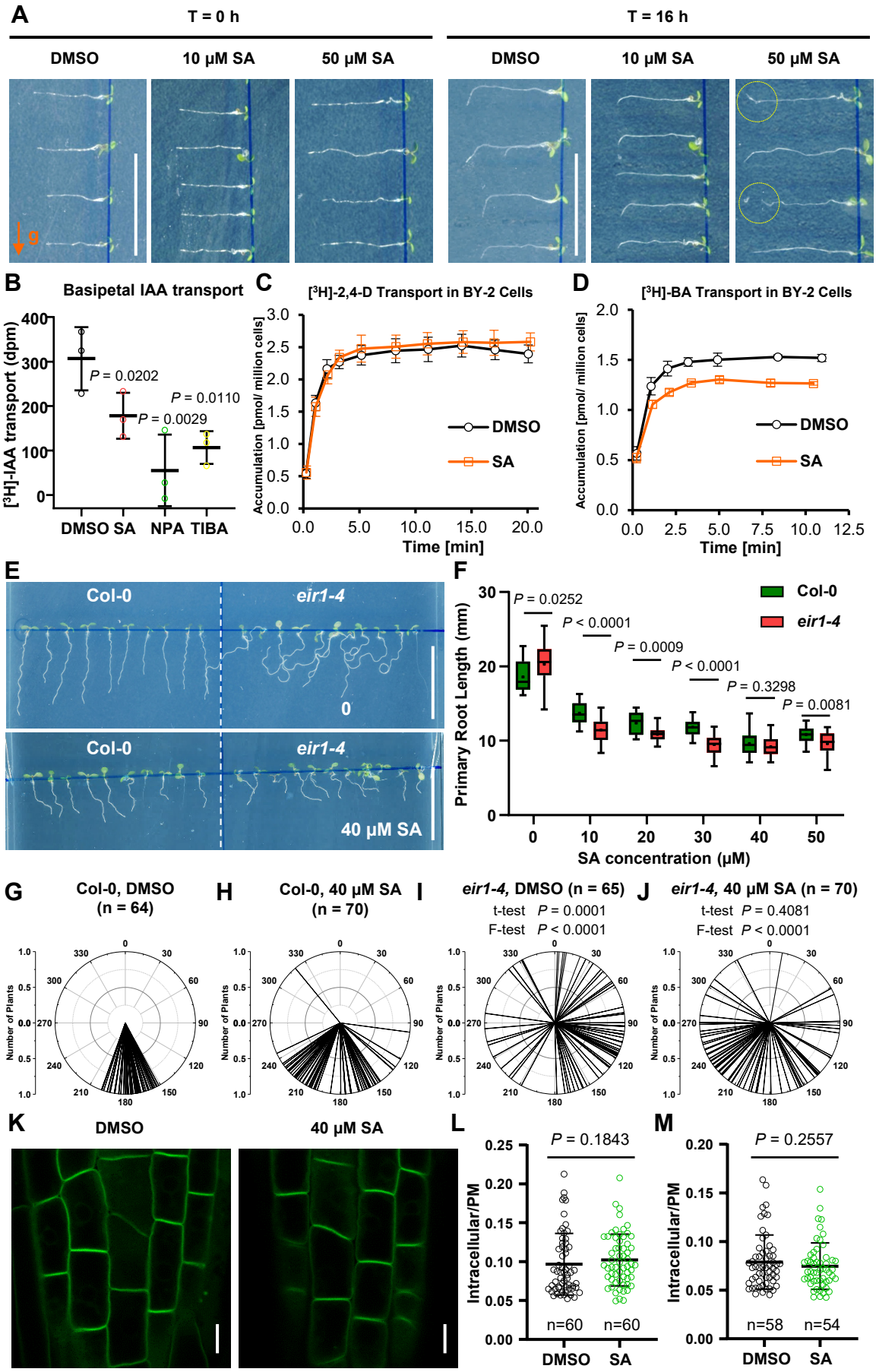

A
T = 0 h
T = 16 h
DMSO
10 µM SA
50 µM SA
DMSO
10 µM SA
50 µM SA
g
B
Basipetal IAA transport
[3H]-IAA transport (dpm)
P = 0.0202
P = 0.0110
P = 0.0029
DMSO SA
NPA
TIBA
C
[3H]-2,4-D Transport in BY-2 Cells
Accumulation [pmol/ million cells]
Time [min]
DMSO
SA
D
[3H]-BA Transport in BY-2 Cells
Accumulation [pmol/ million cells]
Time [min]
DMSO
SA
E
Col-0
eir1-4
0
Col-0
eir1-4
40 µM SA
F
Col-0
eir1-4
Primary Root Length (mm)
P = 0.0252
P < 0.0001
P = 0.0009
P < 0.0001
P = 0.3298
P = 0.0081
SA concentration (µM)
G
Col-0, DMSO
(n = 64)
H
Col-0, 40 µM SA
(n = 70)
I
eir1-4, DMSO (n = 65)
t-test P = 0.0001
F-test P < 0.0001
J
eir1-4, 40 µM SA (n = 70)
t-test P = 0.4081
F-test P < 0.0001
Number of Plants
K
DMSO
40 µM SA
L
P = 0.1843
Intracellular/PM
n=60
n=60
DMSO
SA
M
P = 0.2557
Intracellular/PM
n=58
n=54
DMSO
SA

Figure S2. Specific SA action on root gravitropic response and auxin export, in a PIN-dependent manner. Related to Figure 3.

(A) Representative images showing the gravitropic response of WT seedlings on different concentrations of SA. 5-d-old seedlings were transferred to indicated plates, and grown for additional 16 h. DMSO as the solvent control. Agravitropic roots are marked. Scale bars, 2 cm.

(B) SA inhibited the basipetal transport of [^{3}H]-IAA in etiolated hypocotyls. DMSO, 10 μM NPA, 10 μM TIBA, and 500 μM SA were added to both the [^{3}H]-IAA droplets and the medium. 15 seedlings were pooled as a biological replicate; n = 3. Dots represent individual values, and lines indicate mean ± SD. *P* values were calculated by one-way ANOVA with a Tukey multiple comparison test, compared to DMSO.

(C-D) SA has no effect on the accumulation of [^{3}H]-2,4-D (C) or [^{3}H]-BA (D) in tobacco BY-2 cells, suggesting no effect on export, as controls for [^{3}H]-IAA. DMSO (the solvent control) and 200 μM SA were added to the cell culture, and then the radioactivity of ^{3}H was measured at indicated timepoints after the addition of [^{3}H]-2,4-D (C) or [^{3}H]-BA (D) to the DMSO- and SA-treated cell cultures. n = 3.

(E) Representative images show the sensitivity of *eir1-4* to SA. Col-0 and *eir1-4* seedlings were grown on plates with different concentrations of SA for 7 d. Scale bars, 2 cm.

(F) *eir1-4* shows slightly increased sensitivity to SA in root growth inhibition. Col-0 and *eir1-4* seedlings were grown on plates with concentrations of SA for 7 d, and the primary root length was measured. n = 16-23; *P* values were calculated by a two-tailed t-test for indicated pairs of Col-0 and *eir1-4* at a certain concentration of SA.

(G-J) *eir1-4* showed agravitropic roots, which were not further enhanced by SA treatment. Col-0 and *eir1-4* seedlings were grown on plates with different concentrations of SA for 7 d, and the root tip angles were measured by Image J, and shown by polar bar charts. *P* values were calculated by a two-tailed t-test to evaluate the mean value and by a further F-test to indicate differences of variances. The *eir1-4* groups were compared with Col-0 under treatment with the same concentration of SA respectively.

(K) The localization of AUX1-YFP. Plants were grown for 4 d on DMSO or 40 μM SA. Scale bars, 10 μm.

(L-M) Quantification of AUX1-YFP subcellular distribution revealed by its intracellular/PM fluorescence ratio. The average AUX1-YFP fluorescence of the intracellular area and PM of 5-10 representative cells from 10 seedlings for each treatment was measured by Fiji. The data points were shown as dot-plots. Dots represent individual values, and lines indicate mean ± s.d.. *P* values were calculated by a two-tailed t-test. (L) 4-d-old seedling grown on plates with 40 μM SA are shown in (K); (M) 4-d-old seedling were treated with 40 μM SA for 12 h.

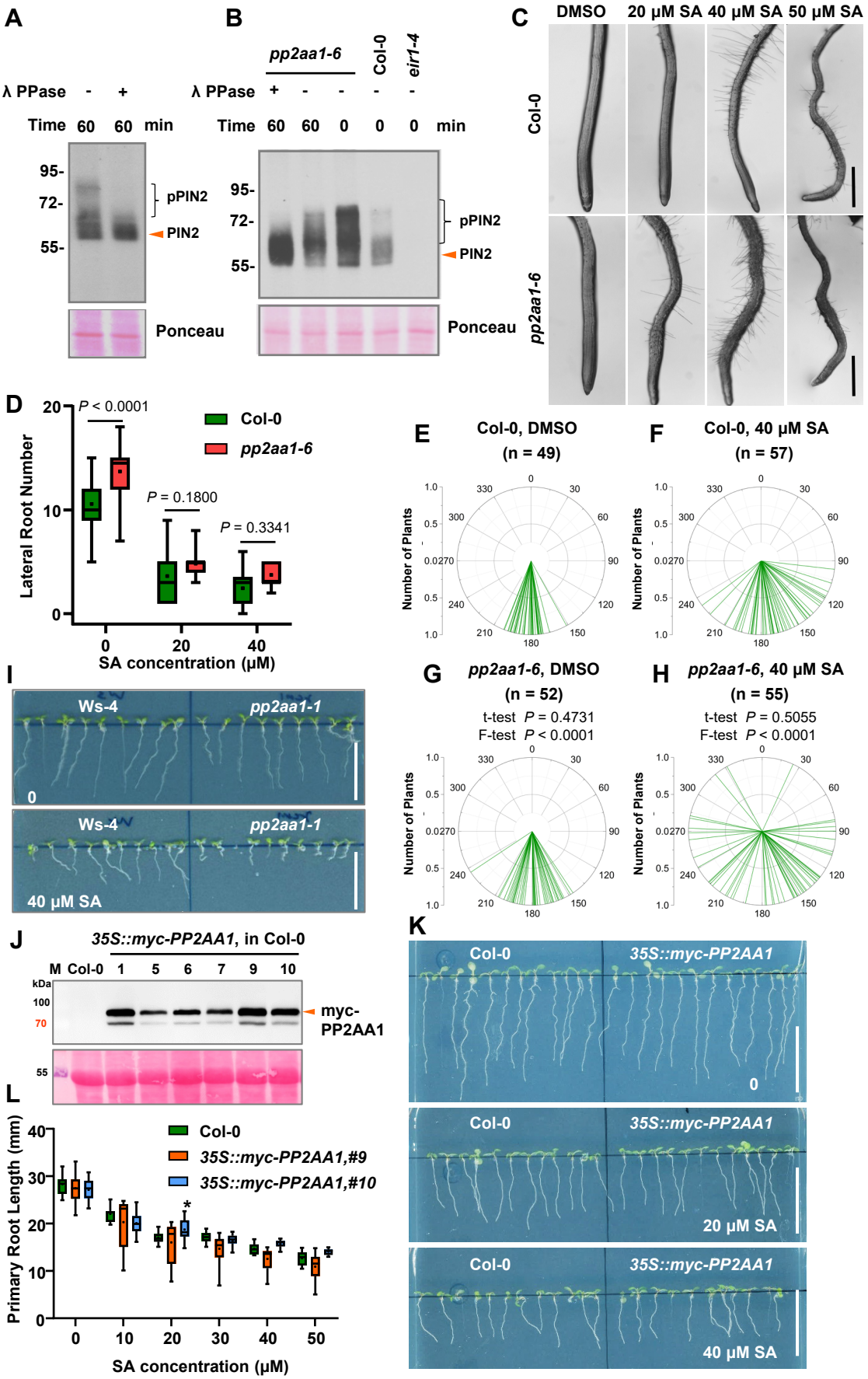

A
λ PPase - +
Time 60 60 min
95-
72-
55-
pPIN2
PIN2
Ponceau
B
pp2aa1-6
Col-0
eir1-4
λ PPase + - - - -
Time 60 60 0 0 0 min
95-
72-
55-
pPIN2
PIN2
Ponceau
C
DMSO
20 μM SA
40 μM SA
50 μM SA
Col-0
pp2aa1-6
D
P < 0.0001
P = 0.1800
P = 0.3341
Col-0
pp2aa1-6
Lateral Root Number
SA concentration (μM)
E
Col-0, DMSO
(n = 49)
F
Col-0, 40 μM SA
(n = 57)
Number of Plants
G
pp2aa1-6, DMSO
(n = 52)
t-test P = 0.4731
F-test P < 0.0001
H
pp2aa1-6, 40 μM SA
(n = 55)
t-test P = 0.5055
F-test P < 0.0001
I
Ws-4
pp2aa1-1
0
40 μM SA
J
35S::myc-PP2AA1, in Col-0
M Col-0 1 5 6 7 9 10
kDa
100
70
55
myc-PP2AA1
K
Col-0
35S::myc-PP2AA1
0
20 μM SA
40 μM SA
L
Col-0
35S::myc-PP2AA1,#9
35S::myc-PP2AA1,#10
Primary Root Length (mm)
SA concentration (μM)

Figure S3. Deficiency of the PP2A A subunit, PP2AA1 (RCN1), leads to SA hypersensitivity, and SA treatment inhibits PP2A activity *in planta*. Related to Figure 4.

(A) PIN2 phosphorylation gave rise to shifted smears, revealed by dephosphorylation of PIN2 by lambda phosphatase (λ PPase) *in vitro*. Total membrane extracts were incubated with or without 2 U of λ PPase for the indicated time, and samples were then analysed by Western blot with an anti-PIN2 antibody. Upper, anti-PIN2; bottom, Ponceau staining.

(B) Increase of PIN2 phosphorylation in *pp2aa1-6*. Total membrane extracts from Col-0, *pp2aa1-6*, and *eir1-4* were incubated with or without 5 U λ PPase for the indicated time, and samples were analysed by Western blot with an anti-PIN2 antibody. Upper, anti-PIN2; bottom, Ponceau staining.

(C) Close views of the morphology of Col-0 and *pp2aa1-6* roots under different concentrations of SA. Seedlings were observed by a differential interference contrast (DIC) microscopy. Scale bars, 1 mm.

(D) Sensitivity of *pp2aa1-6* to SA in terms of inhibiting lateral root formation. Col-0 and *pp2aa1-6* seedlings were grown on plates containing different concentrations of SA for 10 d, and the number of emerged lateral root was counted. *P* values were calculated by a two-tailed t-test.

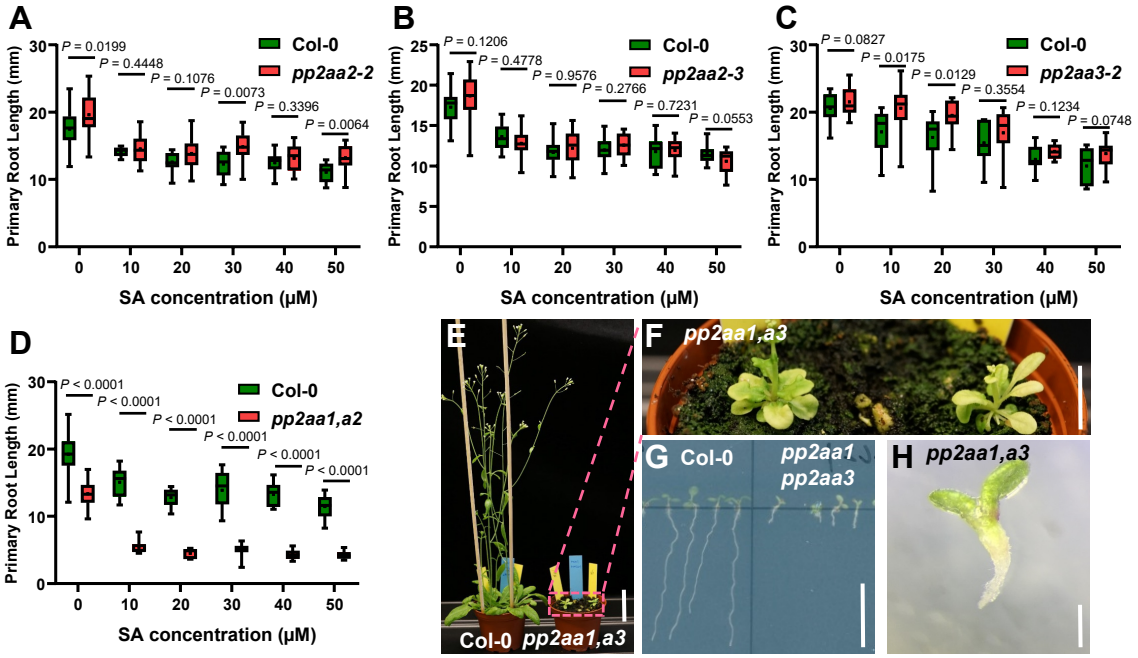
(E-H) Following Figure 4F. *pp2aa1-6* is hypersensitive to SA in terms of interfering with the gravitropic response. Col-0 (E-F) and *pp2aa1-6* (G-H) seedlings were grown on plates containing different concentrations of SA for 7 d, and the root tip angles were measured by Image J, and shown as polar bar charts. *P* values were calculated by a two-tailed t-test to evaluate the mean value and by a further F-test to indicate differences of variances. The *pp2aa1-6* groups were compared with Col-0 under treatment with the same concentration of SA respectively.

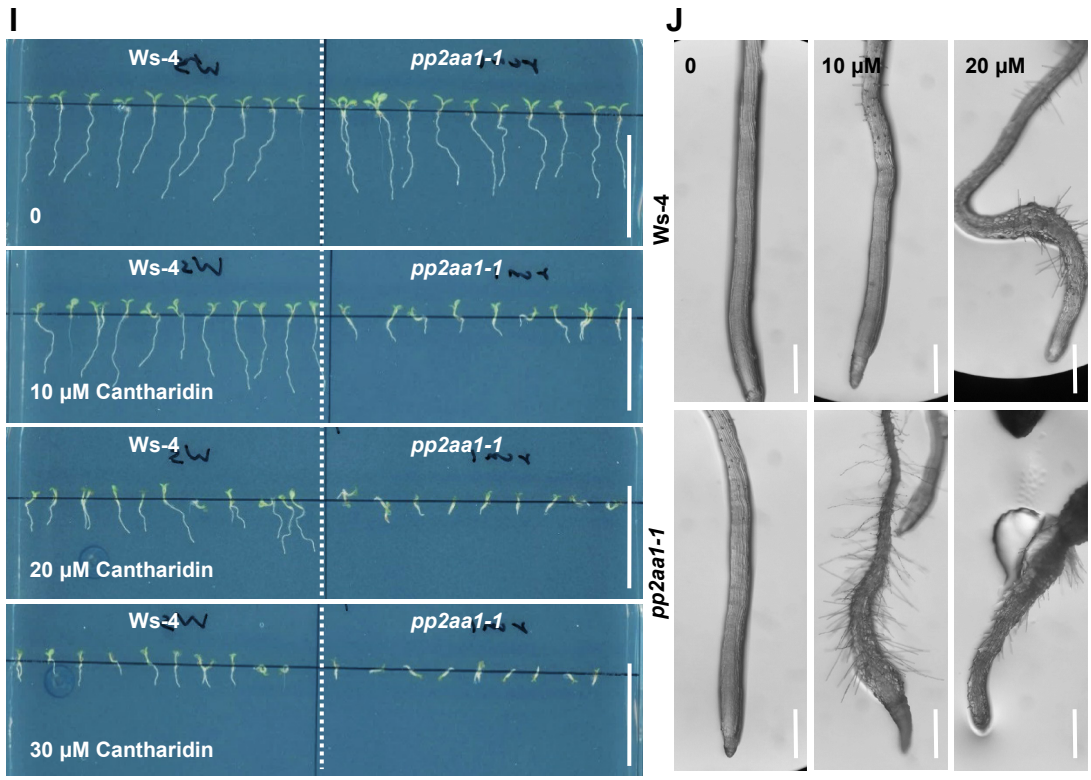
(I) Representative images showing hypersensitivity of *pp2aa1-1* to SA. WT (Ws-4) and *pp2aa1-1* seedlings were grown on plates with different concentrations of SA for 7 d. Scale bars, 2 cm.

(J) Western blot verified the expression of myc-PP2AA1. 7-d-old seedlings were subjected to protein extraction and the subsequent Western blot with an anti-myc antibody (1:2000). Upper panel, anti-myc; lower panel, Ponceau staining to show the loading. Lines 9 and 10 (T_3 generation, homozygous lines) were used for further analysis.

(K) Representative images showing the sensitivity of *35S::myc-PP2AA1* to SA. Col-0 and *35S::myc-PP2AA1* seedlings were grown on plates with different concentrations of SA for 7 d. Scale bars, 2 cm.

(L) *35S::myc-PP2AA1* did not show any difference in sensitivity to SA in root growth inhibition. Col-0 and *35S::myc-PP2AA1* seedlings were grown on plates with concentrations of SA for 7 d, and the primary root length was measured. n = 11-30; *, $P < 0.05$, by one-way ANOVA with a Tukey multiple comparison test, compared to Col-0.


A
Primary Root Length (mm)
Col-0
pp2aa2-2
P = 0.0199
P = 0.4448
P = 0.1076
P = 0.0073
P = 0.3396
P = 0.0064
SA concentration (μM)
B
Col-0
pp2aa2-3
P = 0.1206
P = 0.4778
P = 0.9576
P = 0.2766
P = 0.7231
P = 0.0553
SA concentration (μM)
C
Col-0
pp2aa3-2
P = 0.0827
P = 0.0175
P = 0.0129
P = 0.3554
P = 0.1234
P = 0.0748
SA concentration (μM)
D
Col-0
pp2aa1,a2
P < 0.0001
P < 0.0001
P < 0.0001
P < 0.0001
P < 0.0001
P < 0.0001
SA concentration (μM)
E
Col-0
pp2aa1,a3
F
pp2aa1,a3
G
Col-0
pp2aa1
pp2aa3
H
pp2aa1,a3


I
Ws-4
pp2aa1-1
0
10 μM Cantharidin
20 μM Cantharidin
30 μM Cantharidin
J
0
10 μM
20 μM
Ws-4
pp2aa1-1

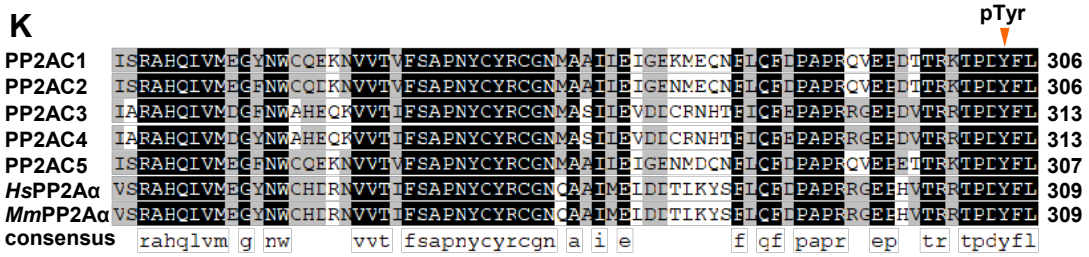

K
pTyr
PP2AC1 306
PP2AC2 306
PP2AC3 313
PP2AC4 313
PP2AC5 307
HsPP2Aα 309
MmPP2Aα 309
consensus

Figure S4. SA sensitivity of the loss-of-function mutants of PP2A subunits, and deficiency of the PP2A A subunit, PP2AA1 (RCN1), leads to hypersensitivity to a known PP2A inhibitor, cantharidin. Related to Figures 4 & 5.

(A-D) The sensitivity of different PP2A mutants to SA in primary root elongation. Col-0, *pp2aa2-2*, *pp2aa2-3* (a knock-down line), *pp2aa3-2*, and *pp2aa1 pp2aa2-3* (short as *pp2aa1,a2*) seedlings were grown on plates with different concentrations of SA for 7 d, and the primary root length was measured. (A) n = 11-27，(B) n = 12-30，(C) n = 10-28，(D) n = 13-28. *P* values were calculated by a two-tailed t-test for indicated pairs of Col-0 and *pp2aa1, a3* at the given concentration of SA.

(E-H) The double mutant of *pp2aa1 pp2aa3* exhibited deficiency in growth and development with severe root defects, reminiscent of SA- or cantharidin- treatment. Scale bar, 5 cm (E), 1 cm (F), 1 cm (G), and 1 mm (H) respectively.

(I) Representative images showing the hypersensitivity of *pp2aa1-1* to cantharidin. Ws-4 and *pp2aa1-1* seedlings were grown on plates containing different concentrations of cantharidin for 7 d. Scale bars, 2 cm.

(J) Close view of the morphology of Ws-4 and *pp2aa1-1* roots under different concentrations of cantharidin. Scale bars, 1 mm.

(K) Sequence alignment of *Arabidopsis* PP2A C subunits, with their homologues in human (*Homo sapiens*) and mice (*Mus musculus*). All the sequences share 87.13% similarity. The arrowhead indicates the conserved phosphorylation site, which is responsible for PP2Ac activity and is recognized by the pY307-PP2Ac antibody.

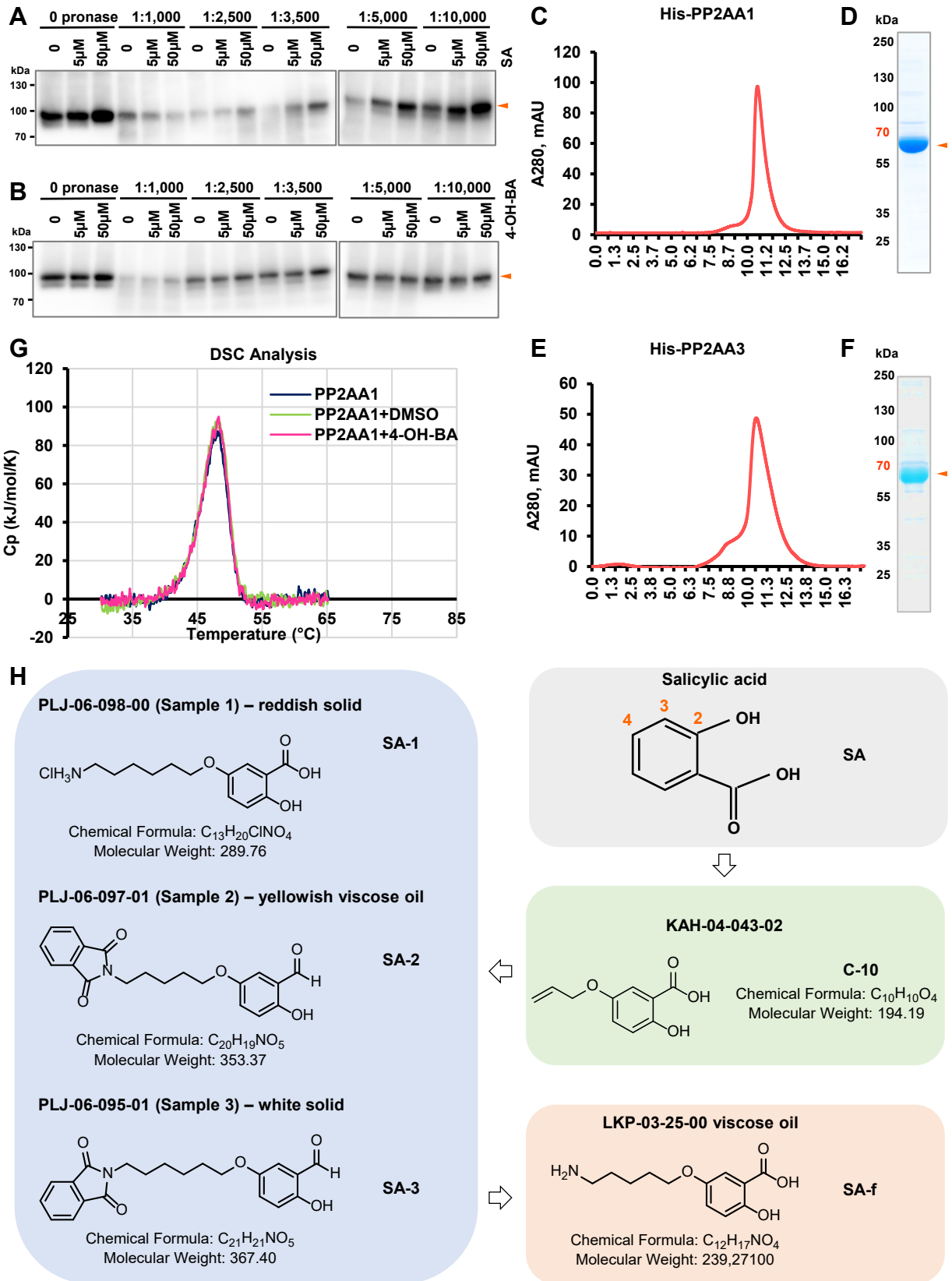

A
0 pronase
1:1,000
1:2,500
1:3,500
1:5,000
1:10,000
0
5μM
50μM
kDa
130
100
70
SA
B
4-OH-BA
C
His-PP2AA1
A280, mAU
D
kDa
250
130
100
70
55
35
25
E
His-PP2AA3
F
G
DSC Analysis
PP2AA1
PP2AA1+DMSO
PP2AA1+4-OH-BA
Cp (kJ/mol/K)
Temperature (°C)
H
PLJ-06-098-00 (Sample 1) – reddish solid
SA-1
Chemical Formula: C13H20ClNO4
Molecular Weight: 289.76
PLJ-06-097-01 (Sample 2) – yellowish viscose oil
SA-2
Chemical Formula: C20H19NO5
Molecular Weight: 353.37
PLJ-06-095-01 (Sample 3) – white solid
SA-3
Chemical Formula: C21H21NO5
Molecular Weight: 367.40
Salicylic acid
SA
KAH-04-043-02
C-10
Chemical Formula: C10H10O4
Molecular Weight: 194.19
LKP-03-25-00 viscose oil
SA-f
Chemical Formula: C12H17NO4
Molecular Weight: 239,27100

Figure S5. DARTS assay suggests potential binding of SA to PP2AA1, protein purification of His-PP2AA1 and His-PP2AA3 by Size exclusion chromatography (SEC), and the design flow of the SA analogue, SA-f, for the SPR. Related to Figure 6.

(A-B) DARTS assay suggests PP2AA1 is potential target of SA. *pPP2AA1::PP2AA1-GFP* seedlings were used for the protein isolation, and Samples were treated with DMSO (mock) and SA (0, 5 μM, and 50 μM respectively, in A), with 4-OH-BA as a negative control (B), and digested by different concentrations of pronase.

(C) SEC purification of His-PP2AA1 with a Superdex 200 increase column. A representative run is shown here to indicate the purity of recombinant His-PP2AA1 used for DSC and SPR analyses.

(D) SDS-PAGE to check the quality of His-PP2AA1, visualized by CBB staining.

(E) SEC purification of His-PP2AA3.

(F) SDS-PAGE to check the quality of His-PP2AA3, by CBB staining.

(G) DSC analysis of the effect of 4-OH-BA, an inactive SA isomer, on His-PP2AA1 stability. 5 μM of purified His-PP2AA1 protein were added with or without DMSO, or 50 μM 4-OH-BA, and were then analysed by DSC. Tm = 47.57°C, 47.69°C, and 47.69°C, for His-PP2AA1, His-PP2AA1+DMSO, and His-PP2AA1+4-OH-BA respectively.

(H) Workflow for the design of the synthetic SA analogue, SA-f, which can be immobilized on a CM-5 SPR sensor chip. Multiple SA analogues with different groups at different positions of the benzoic ring were synthesized, tested, with C-10 coming out as the best one with activity and the possibility to immobilize it. Further analysis for SA-1/2/3 with a linker and a group to mimic the surface of the matrix of SPR sensor chips, revealed that the linker does not affect the bioactivity of SA.

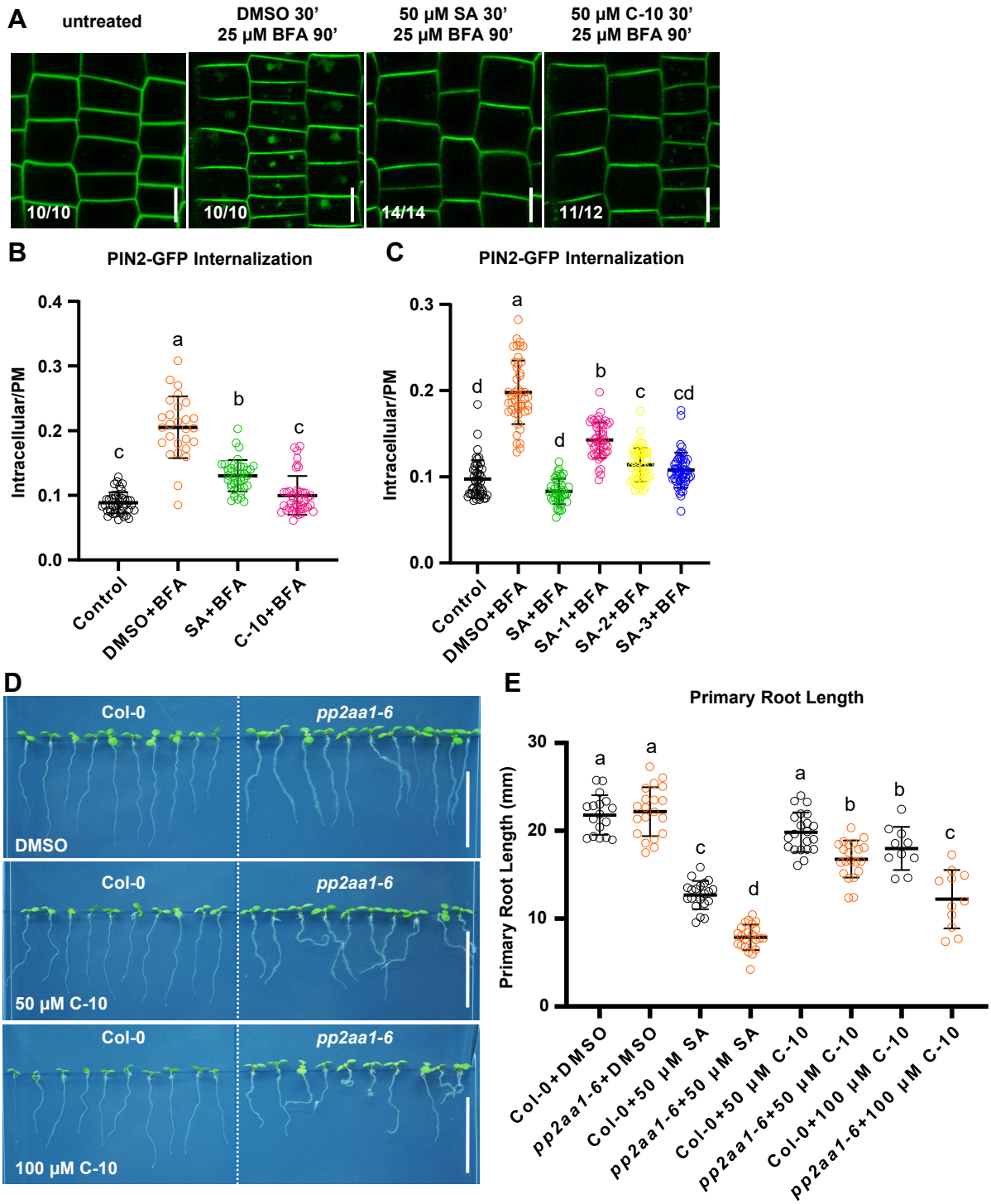

A
untreated
DMSO 30'
25 μM BFA 90'
50 μM SA 30'
25 μM BFA 90'
50 μM C-10 30'
25 μM BFA 90'
10/10
10/10
14/14
11/12
B
PIN2-GFP Internalization
Intracellular/PM
Control
DMSO+BFA
SA+BFA
C-10+BFA
C
PIN2-GFP Internalization
Intracellular/PM
Control
DMSO+BFA
SA+BFA
SA-1+BFA
SA-2+BFA
SA-3+BFA
D
Col-0
pp2aa1-6
DMSO
50 μM C-10
100 μM C-10
E
Primary Root Length
Primary Root Length (mm)
Col-0+DMSO
pp2aa1-6+DMSO
Col-0+50 μM SA
pp2aa1-6+50 μM SA
Col-0+50 μM C-10
pp2aa1-6+50 μM C-10
Col-0+100 μM C-10
pp2aa1-6+100 μM C-10
F
DMSO (n = 60)
50 μM C-10 (n = 48)
t-test P = 0.2521
F-test P < 0.0001
DMSO (n = 61)
50 μM C-10 (n = 47)
t-test P = 0.3365
F-test P < 0.0001
t-test P = 0.4076
F-test P < 0.0001
Col-0
pp2aa1-6
Number of Plants

Figure S6. Bioactivity test of the synthetic SA analogues. Related to Figure 6.

(A-B) Cellular activity of the synthetic compound, C-10, with the SA moiety, in terms of inhibiting BFA (brefeldin A) body formation. 4-d-old *pPIN2::PIN2-GFP* seedlings were treated with indicated chemicals and imaged by CLSM. (A), representative images, Scale bars, 10 μm; (B), quantification of the BFA body formation by calculating the intracellular/PM ratio for the PIN2-GFP fluorescence intensity.

(C) Cellular activity of the synthetic compounds, SA-1 to SA-3, in terms of inhibiting BFA body formation. 4-d-old *pPIN2::PIN2-GFP* seedlings were treated with indicated chemicals and imaged by CLSM. Quantification of the BFA body formation by calculating the intracellular/PM ratio for the PIN2-GFP fluorescence intensity.

Dots represent individual values, and lines indicate mean ± SD. (B) n = 27-42; (C) n = 41-50. Different letters represent significant difference, $P < 0.05$, by one-way ANOVA with a Tukey multiple comparison test.

(D) Physiological activity of the synthetic SA analogue C-10, in terms of root morphology. 7-d-old Col-0 seedlings were grown on plates with indicated chemicals. Scale bars, 2 cm.

(E) C-10 inhibits primary root elongation. Dots represent individual values, and lines indicate mean ± SD. n =10-23; *P* values were calculated by a two-tailed t-test.

(F) Treatment with C-10 gave rise to less gravitropic roots. The angles of root tips were measured by Image J, and shown as polar bar charts. *P* values were calculated by a two-tailed t-test to evaluate the mean value and by a further F-test to indicate differences of variances. For Col-0, C-10 treatments were compared with the DMSO control, and the *pp2aa1-6* groups were compared with Col-0 under treatment with the same concentration of C-10 respectively.

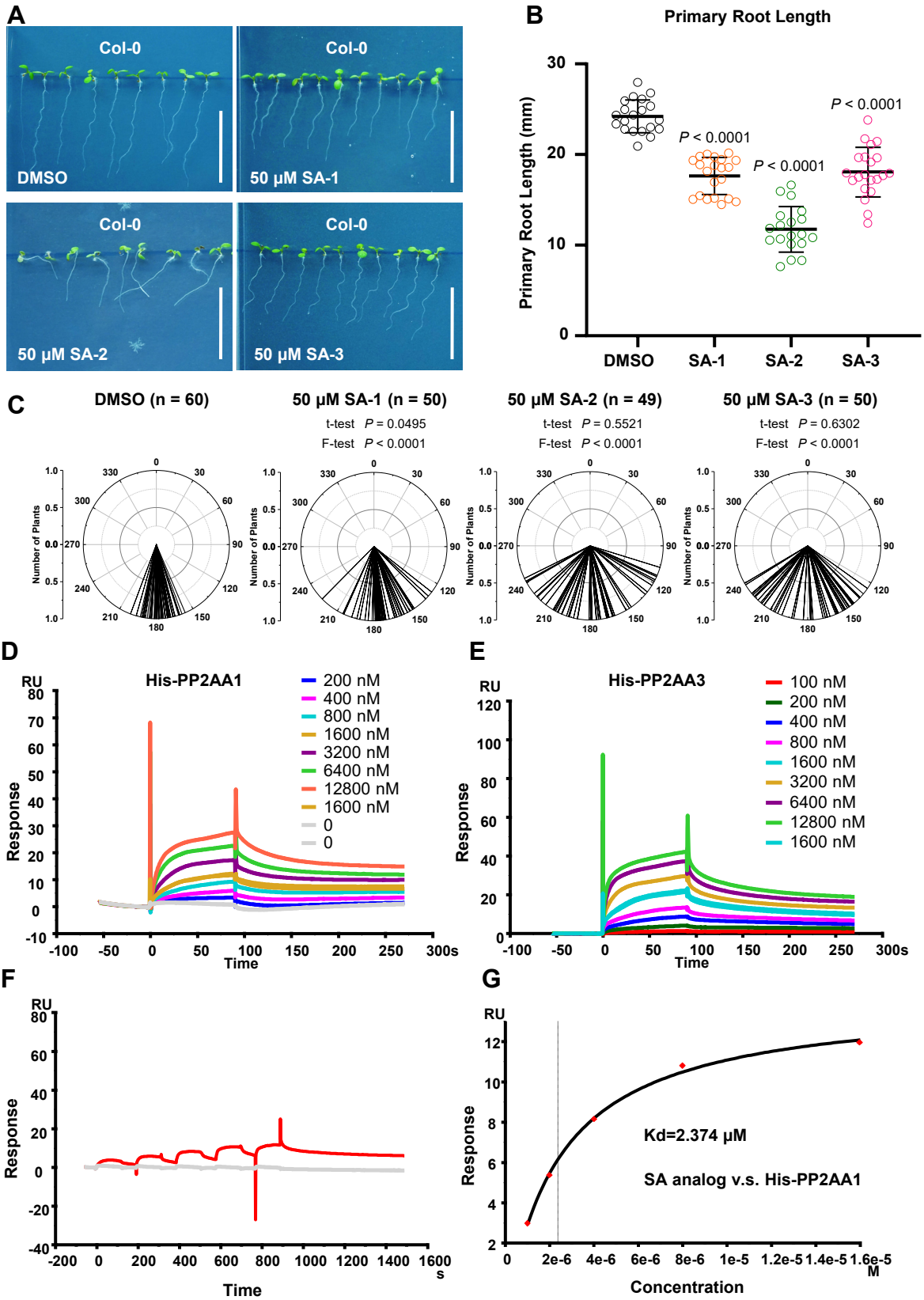

A
Col-0
DMSO
Col-0
50 μM SA-1
Col-0
50 μM SA-2
Col-0
50 μM SA-3
B
Primary Root Length
Primary Root Length (mm)
P < 0.0001
P < 0.0001
P < 0.0001
DMSO
SA-1
SA-2
SA-3
C
DMSO (n = 60)
50 μM SA-1 (n = 50)
t-test P = 0.0495
F-test P < 0.0001
50 μM SA-2 (n = 49)
t-test P = 0.5521
F-test P < 0.0001
50 μM SA-3 (n = 50)
t-test P = 0.6302
F-test P < 0.0001
Number of Plants
D
His-PP2AA1
RU
Response
Time
200 nM
400 nM
800 nM
1600 nM
3200 nM
6400 nM
12800 nM
1600 nM
0
0
E
His-PP2AA3
RU
Response
Time
100 nM
200 nM
400 nM
800 nM
1600 nM
3200 nM
6400 nM
12800 nM
1600 nM
F
RU
Response
Time
G
RU
Response
Kd=2.374 μM
SA analog v.s. His-PP2AA1
Concentration
M

Figure S7. Physiological test of the SA analogues, and additional data for the binding between SA and His-PP2AA1. Related to Figure 6.

(A) Physiological activity of the synthetic SA analogues SA-1 to SA-3, in terms of root morphology. 7-d-old Col-0 seedlings were grown on plates with indicated chemicals. Scale bars, 2 cm.

(B) SA analogues (SA-1 to SA-3) inhibit the primary root elongation. Dots represent individual values, and lines indicate mean ± SD. n =17-21; *P* values were calculated by a two-tailed t-test.

(C) Treatment with SA analogues (SA-1 to SA-3) gave rise to less gravitropic roots. The angles of root tips were measured by Image J, and shown as polar bar charts. *P* values were calculated by a two-tailed t-test to evaluate the mean value and by a further F-test to indicate differences of variances in comparison to mock treatment.

(D) SPR assay reveals the binding of His-PP2AA1 to SA. The sensorgram shows the kinetics for the interaction between His-PP2AA1 and SA, used for generating Figure 6C.

(E) SPR assay reveals the binding of His-PP2AA3 to SA. Sensorgram showing the kinetics for the interaction between His-PP2AA3 and SA.

(F-G) SPR revealing the binding between SA and His-PP2AA1. Single cycle binding kinetics was conducted, without regeneration between different concentrations of His-PP2AA1. 0.1% BSA (~15 μM) was included in the His-PP2AA1 flow. (F), sensorgram; (G), plotted by the values at steady state. A Kd value of 2.374 μM was detected.

Primers	**Oligonucleotide (5’ to 3’)**	**use**
For Genotyping		
Oligo Name	**Sequence**	**Mutants**
pp2aa1-1 _LP	AGCACATCCTTCCTTGTGTGAAGG	*pp2aa1-1*
pp2aa1-1 _RP	AACTTGCTTATGATGTTAAGGCGC	
pp2aa1-1 _RB	TGTCCCGCGTCATCGGCGGGGGTC	
*pp2aa1-6*_LP	GGCCAGCCAGTTAGGTATAGG	*pp2aa1-6*
*pp2aa 1-6*_RP	AAACATAGCCACACGCATTTC	
*pp2aa2-1*_LP	CGATGTTACGTGCCCTCTTAC	*pp2aa2-1*
*pp2aa2-1*_RP	TCTACCGAATGACCATTTTGC	
*pp2aa2-3*_LP	ATTGGTTATTTGGGATCGGAG	*pp2aa2-3*
*pp2aa2-3*_RP	ACTCTCCCTCATCTGAGAGCC	
*pp2aa3-1*_LP	TATTTCCAAACTTTGGGGGAC	*pp2aa3-1*
*pp2aa3-1*_RP	ATGGACACAGCTTGAAGATGG	
*pp2aa3-2*_LP	GCACCAAGCTTCTCATCAAAG	*pp2aa3-2*
*pp2aa3-2*_RP	GACCGGAGCCAACTAGGTAAG	
LB1	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	SAIL lines
LBb1.3	ATTTTGCCGATTTCGGAAC	SALK lines
pp2ac3-1-LP	GCTTGAAAGAACAGCATTTCG	*pp2ac3-1*
pp2ac3-1-RP	GTGGATTATCACCATCCATCG	
pp2ac4-1-LP	TAATTGGTATCAGGGCACTGC	*pp2ac4-1*
pp2ac4-1-RP	TGTTTCCTGATCTGTTTTCCG	
npr1-1-F	CGTGTGCTCTTCATTTCGCTGT	*npr1-1*
npr1-1-R	GTGCGGTTCTACCTTCCAAAGTT	
npr3-1-F	GAGTCAGATATCACTCTAGATCAAGC	*npr3-1*
npr3-1-R	GGAAAGAACAACTGAGCAAGCCCCA	
npr4-3-F	CGGATCTTGTTTCGTCATTTCAG	*npr4-1*
npr4-3-R	CAAACGTGAAATCTGAAGCATTAGC	
sid2-3-LP	ACCCTAATTTGGATTTGGTGC	*sid2-3*
sid2-3-RP	AGCTCTAGGCCTAGTTGCAGC	
For Cloning		
Oligo Name	**Sequence**	**Plasmid**

PIN2HL-1(EcoRI)	TGGAATTCGCTAAGCTTCTCATCTCCGAGC	pET28a-PIN2HL
PIN2HL-2(SalI)	CCGGTCGACACTCGCCGGCGGCATCTGCTG	
PP2AA1-1(EcoRI)	GGAATTCATGGCTATGGTAGATGAACCGTTG	pET28a-PP2AA1
PP2AA1-2(XhoI)	CCGCTCGAGGGATTGTGCTGCTGTGGAACCATC	
PP2AA3-1(EcoRI)	GGAATTCATGTCTATGGTTGATGAGCCTTTA	pET28a-PP2AA3
PP2AA3-2(XhoI)	CCGCTCGAGGCTAGACATCATCACATTGTC	

Table S1. List of primers used in this study. Related to STAR METHODS and Key Resources Table.